

The University of Chicago

**GONAD DIFFERENTIATION IN THE
CHICK EMBRYO AS STUDIED IN
HETEROSEXUAL GRAFT AND
HOST-GRAFT COMBINATIONS**

**A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL
SCIENCES IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY**

DEPARTMENT OF ZOÖLOGY

1934

By

EARL AUBREY DENNIS

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GONAD DIFFERENTIATION IN THE CHICK EMBRYO AS STUDIED IN HETEROSEXUAL GRAFT AND HOST-GRAFT COMBINATIONS¹

(Two plates)

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LILLIE (1917) reported that the embryonic sex glands of the female member of a pair of opposite-sexed twin calves are modified in the male direction as the result of a fusion of its chorionic blood vessels with those of its male co-twin. Several investigators have since employed the chorioallantoic grafting technique as a means of incorporating two gonads of opposite sex in the same embryonic blood circulation of the chick embryo. Such a host-graft relationship would seem to be a suitable method of testing the influence of a gonad graft upon the reproductive organs of the host embryo, or of the gonads of the host upon the engrafted gonad.

Previous experiments involving the transplantation of gonad tissue to the chorioallantoic membrane have been of two general types: (1) Gonad rudiments were transplanted to host embryos whose sex glands were already morphologically differentiated to determine what influence the sex of the host might have upon the capacity of the rudiment to produce a gonad of specific sex (Willier, 1925 and 1927, and Corinaldesi, 1927*a* and 1927*b*). (2) Sexually differentiated gonad tissue isolated from embryos and from juvenile or adult fowl were transplanted to host embryos whose sex glands were in an early stage of morphological sex differentiation (7-9 days' incubation). The latter type of experiment was carried out by Minoura (1921), Greenwood (1925), Kemp (1925 and 1927), and Willier (1927) to determine whether or not the gonad graft influences the process of differentiation in the sex glands and ducts of the host. With the exception of those reported by Minoura, these experiments were negative on the question of sex hormone action in the chick.

¹ This work was carried out at the suggestion and under the direction of Dr. B. H. Willier, to whom I am greatly indebted for helpful suggestions and valuable aid.

Consequently, a further study, employing different methods of approach, seemed advisable.

The first series of experiments reported here was designed to test the self-differentiating capacity of the morphologically undifferentiated gonad when associated for 8 or 9 days in the same chorio-allantoic graft with differentiated gonad tissue (isolated from birds ranging in age between 12 days' incubation and $3\frac{1}{2}$ months' post-hatching). This type of experiment enables one to test possible effects of contiguous or closely associated cortical and testicular tissues upon the direction of differentiation of the indifferent gonad rudiment. Such a relationship may be of primary importance in influencing the course of sex differentiation, as Witschi (1931 and 1934) has suggested for amphibians.

Certain combination grafts in this series contain gonad tissue known to be producing hormone at the time of implantation, i.e., from hatched chicks. Such grafts should throw some light on the question of the capacity of sex hormones which condition secondary sexual characteristics to function also as sex-differentiating hormones.

The second group of experiments was performed to ascertain what influence gonads, already structurally differentiated as to sex, and of the same age, may exert upon each other in heterosexual combination in the same graft. Testes and ovaries were isolated from donors of approximately 18 days of incubation and transplanted in heterosexual pairs to a host embryo, where they developed in close proximity or in direct contact with one another for a period of 8 or 9 days.

The third set of experiments was performed in an endeavor to elicit a response on the part of the reproductive organs of the host embryo to large amounts of gonad tissue transplanted to the chorio-allantoic membrane. So-called "multiple-testis" grafts were made in an attempt to effect in the host embryo a high dosage of male hormone, thereby possibly inducing modification of its reproductive organs by increasing the stimulus above a certain threshold.

MATERIAL AND METHODS

Donor material composing the grafts consisted of: (1) chick gonads morphologically undifferentiated as to sex, and (2) morphologically differentiated gonad tissue. The undifferentiated gonad

rudiments were obtained by isolating separately the right and left urinogenital ridges from embryos incubated between $4\frac{1}{2}$ and 6 days. This operation was performed under sterile conditions in a Petri dish containing warm physiological saline. The differentiated gonad tissue was secured from embryos during the latter half of their incubation period or from hatched birds as old as $3\frac{1}{2}$ months. Immediately after removal, this tissue was placed into warm, sterile salt solution. The grafting technique was essentially the same as that described by Willier (1924 and 1927). The tissue or tissues chosen to compose the graft were removed from the salt solution on the point of a glass needle and placed at the junction of two blood vessels on the chorioallantoic membrane of a previously prepared 8- or 9-day host embryo.

When a right gonad rudiment was combined with differentiated gonad tissue in a combination graft, the left one was transplanted alone to the membrane of a second host, thus serving as control; and vice versa. According to Willier (1927), the morphologically indifferent gonad has the capacity to self-differentiate according to its original plan of organization regardless of the sex of its host. Such a control graft thus affords a means of ascertaining the originally determined sex of its partner in the combination graft.

Eight or 9 days after operation the grafts were removed, fixed in Bouin's fluid, sectioned at 7μ and stained with iron haematoxylin. The reproductive organs of all hosts were closely examined under a binocular microscope for evidence of any atypical structure. The ovaries of female hosts bearing "multiple-testis" grafts were examined histologically. A number of control grafts of testicular or ovarian tissue, as well as normal ovaries and testes of an age corresponding to those contained in the combination grafts, were frequently referred to during the examination of the experimental material. Eggs and tissues from the white Leghorn breed of fowl were used exclusively in these experiments.

ASSOCIATION OF AN INDIFFERENT GONAD RUDIMENT WITH DIFFERENTIATED GONAD TISSUE

A. THEORETICAL NUMBER OF GONAD COMBINATIONS

At the time the structurally indifferent gonad primordium (4-6 days' incubation) is transplanted with older testicular or ovarian tissue, only its age and laterality are known. During the 8 or 9 days

on the membrane the rudiment undergoes sex differentiation. To test its self-differentiating capacity in the presence of older gonad tissue, eight types of double transplants were made: right or left gonad rudiments, structurally undifferentiated as to sex, were transplanted with a piece of a right or left ovary or a right or left testis. The control in each case was the opposite gonad rudiment transplanted alone to a second host.

If the structurally undifferentiated gonad has the capacity to self-differentiate in accordance with its originally determined sex, and the laterality of each component of the graft is considered, sixteen possible combinations of gonad tissue should arise from the eight types of double transplants. Considering the marked and significant difference in structure and function between the left and the right ovary in the domestic fowl, it was essential to record the laterality of all ovarian tissue transplanted. Since, on the other hand, no such differences exist between the right and left testis in late embryonic, juvenile, or adult birds, the number of possible gonad combination may be reduced to twelve if the laterality of the testicular tissue transplanted is disregarded.

If the sex of the host is considered, twenty-four possible gonad relationships exist, twenty-three of which have been obtained in these experiments. It was found that each of the twelve possible gonad combinations was represented in the combination grafts, following the sex differentiation of the structurally indifferent component. In a number of cases the gonad rudiment which served as a control to its partner in a combination graft was lost. This is to be expected, since death of the host frequently follows the operation. In eighty-three out of the total one hundred and twenty-three cases the gonad rudiment in both the combination and the control graft survived. Table I shows the twelve possible types of combination graft, the number of cases recovered representing each type, and their relation to the sex of the host. Those in which the gonad rudiment gave rise to a gonad-like body, unspecific as to sex, are also recorded.

B. THE MORPHOLOGY OF THE "INDIFFERENT" GONAD PRIMORDIUM AT THE TIME OF IMPLANTATION

The majority of gonad primordia transplanted in combination grafts were isolated from donor embryos which had been incubated

for 5 or $5\frac{1}{2}$ days. Two were as old as 6 days; fourteen were 4 and a fraction days of age.

At the end of the fourth day of incubation, according to Swift (1915), the developing gonad consists of a whitish ridge approximately 1.5 mm. in length lying on the ventromedial surface of the Wolffian body. It is composed of three types of tissue: (1) a germinal epithelium, two or three cell layers in thickness; (2) a compact mass of embryonic mesenchyme which lies beneath the germinal

TABLE I

ASSOCIATED DIFFERENTIATED GONAD	SEX OF HOST	RIGHT UNDIFF- ERENTIATED GONAD			LEFT UNDIFFERENTIATED GONAD			
		Right Ovary	Right Testis	Right Not Ascer- tained	Left Ovary		Left Testis	Left Not Ascer- tained
					Typical	Atypical		
Left ovary.....	♀	3	2	1	4	1	2	0
	♂	2	2	0	1	0	4	1
Right ovary....	♀	3	1	0	4	0	5	0
	♂	2	0	1	1	3	5	1
Testis.....	♀	1	4	0	7	1	14	4
	♂	4	2	3	4	14	11	5
Total.....		15	11	5	21	19	41	11

epithelium; and (3) primordial germ cells, which are found between the epithelial cells of the germinal epithelium, as well as lying deeper in the embryonic mesenchyme. It is an interesting fact that the number of primordial germ cells at this early stage of development is greater in the left gonad rudiment than in the right (Firket, 1914; Swift, 1915).

At approximately the 132d hour of incubation the primary sex cords (cords of first proliferation) begin to invaginate into the underlying mesenchyme from the germinal epithelium. This process continues for the next 24 hours, certain of the sex cords in the meantime making connection with certain Malpighian corpuscles of the Wolffian body by means of the rete cords (cords of urinogenital union). At $6\frac{1}{2}$ days (156 hours) the proliferation of the primary sex cords rather

abruptly ceases and sexual differences become morphologically apparent.

In these experiments, therefore, the gonad primordia contributing to the combination grafts or transplanted singly as controls were at the time of implantation "morphologically indifferent" as to sex.

C. SEX OF GONADS DIFFERENTIATING FROM GRAFTED RUDIMENTS

At 14 or 15 days of incubation the gonad of the chick has a characteristic histological structure which enables one to determine the sex, and, in the case of the female, the laterality. With certain exceptions, which will be described later, the gonad rudiments which gave rise to gonads in combination grafts possess a similar characteristic histological structure.

The typical left ovary found in a combination graft has two markedly different structural components. It possesses both a cortex and a medulla (Plate I, Fig. 3). The medulla is composed of a diffuse network of epithelial cords. The cortex is more compact in appearance and is made up of cords of epithelial and germ cells which have invaginated from the germinal epithelium, at this time still composed of columnar epithelial cells and a few germ cells. This cortical layer overlies the medulla and covers the entire surface of the ovary with the exception of the area where the gland is attached to the Wolffian body. In short, the appearance of the ovary is strikingly similar to a normal left ovary of corresponding age.

The right ovary found in a combination graft differs from the typical left ovary in three principal characteristics: (1) its much smaller size; (2) absence of a set of cortical cords; and (3) a flat, inactive epithelial covering (Plate I, Fig. 1).

A testis which arises from a gonad rudiment in a combination graft can be easily distinguished from either the left or the right ovary since it possesses a well-developed tunica albuginea and a set of elongated sex cords whose structural components—the sex cells and supporting epithelial cells—are arranged in a characteristic spacial relationship within the cord (Plate I, Fig. 2). The identification of sex of all gonads arising from transplanted "indifferent" gonad rudiments was made on the basis of the above-mentioned criteria.

PLATE I

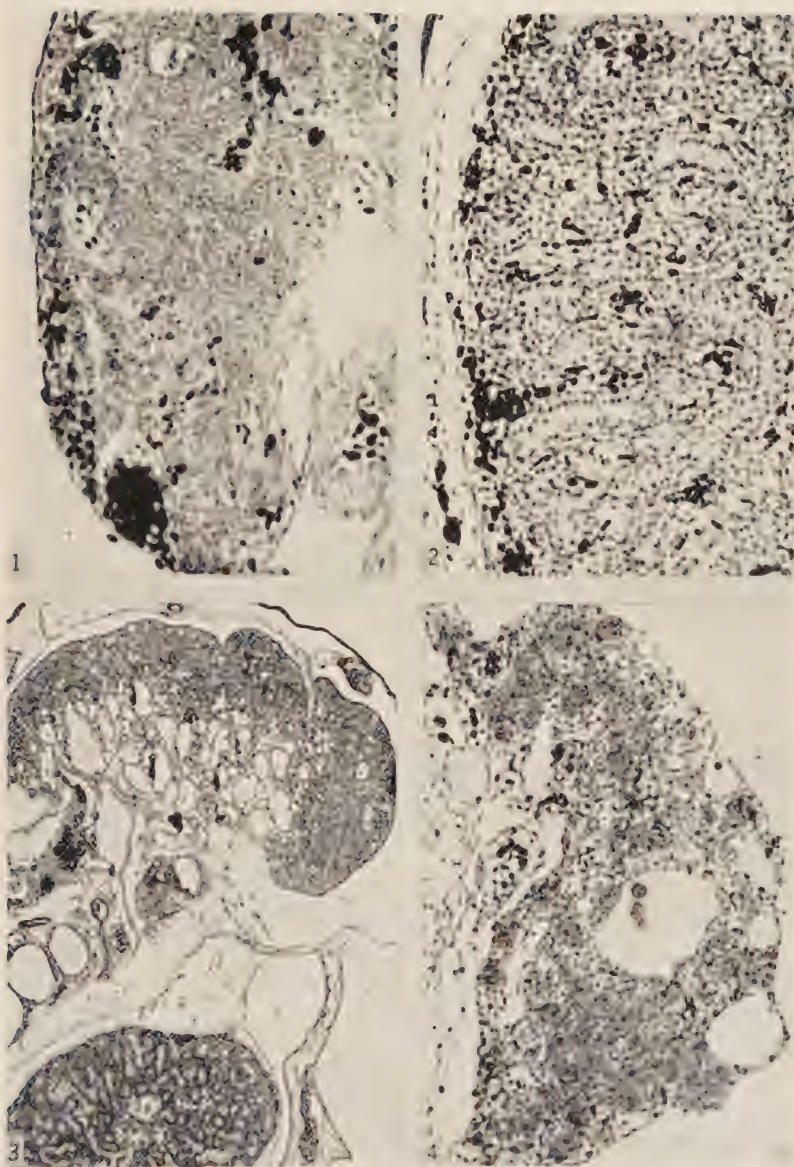
FIG. 1.—Section through a right ovary which differentiated from a right gonad rudiment combined with testicular tissue from a 51-day-old cock; grown for 9 days on a male host. $\times 500$.

FIG. 2.—Section through a testis derived from a right gonad rudiment combined with a left ovary from an embryo of $12\frac{1}{2}$ days' incubation and grown on a female host. $\times 400$.

FIG. 3.—Longitudinal section through a left ovary differentiated from a left gonad rudiment in combination with a 17-day embryonic testis; 9 days on a male host. Note well-differentiated medulla and cortex. $\times 65$.

FIG. 4.—Section of a modified left ovary derived from a left gonad rudiment, combined with an 18-day right ovary and grown on a male host. $\times 315$.

PLATE I



The older gonad tissue which was transplanted with the sexually undifferentiated rudiment is strikingly different from the young sex gland which has undergone differentiation during the growth period of the graft. The difference in age is associated with sharp histological differences in structure, thus facilitating identification of the two components. Even in those cases where the sex of the younger gonad and the older gonad tissue is the same, no opportunity for confusing the two exists.

D. DIFFERENTIATION OF THE RIGHT "INDIFFERENT" GONAD PRIMORDIUM

Thirty-one grafts were examined in which a right "indifferent" gonad rudiment was combined with ovarian or testicular tissue. Fifteen differentiated into right ovaries, eleven into testes, and five into gonad-like bodies whose sex could not be ascertained (see Table I).

1. *Right ovaries*.—Five right ovaries differentiated in grafts with left ovarian tissue (from embryos between 13 and 19 days' incubation), three of which developed on female and two on male hosts. Five were combined with right ovaries from embryos of 18 days' incubation, three of which developed on female and two on male hosts. Of the remaining five, which developed with testicular tissue from chicks ranging in age between 17 days' incubation and 80 days' post-hatching, four developed on male, and one on a female, host.

Regardless of the nature of the combination, the right ovary, which differentiates from the right indifferent gonad, is small and quite similar to the normal in general appearance. In the greater number of cases it consists of medullary elements only, although occasionally it is found to possess a few scattered areas of cortical tissue. That the presence of these cortical elements is not due to any specific factor of the environment operating during the development of the graft is strongly suggested by their rather frequent appearance in normal right ovaries (Brode, 1928).

In the majority of cases the right ovary is a more or less compact, definitely circumscribed, elongated body, broadly attached to the mesonephros (Plate I, Fig. 1). Occasionally, however, it may have a loose texture and present the appearance of a stromal network. Histologically, it consists of cords of epithelial or primordial germ

cells imbedded in a connective tissue stroma. Near the point where the ovary is attached to the Wolffian body, distention of the medullary cords frequently occurs. In the lumina of such distended cords epithelial cells or primordial germ cells may be found, either singly or in nests consisting of several cells. The surface of the right ovary lacks a distinct germinal epithelium and is covered by a single layer of flattened cells. Even in those right ovaries in which scattered cortical elements are present, the overlying germinal epithelium is flattened and inactive. This inactive condition of the germinal epithelium is also characteristic of the normal right ovary (Firket, 1920).

On theoretical grounds it is expected that the right ovary, known to possess male potentialities, might be modified in the male direction under the stimulation of hormones from older testicular tissue. In five cases right ovaries differentiated from right gonad rudiments which grew in the same graft with testicular tissue obtained from donors ranging in age from 17 days' incubation to 80 days' post-hatching. Four of these grafts grew on male hosts; one, on a female host. That these gonad rudiments were really determined as right ovaries is shown by the fact that, in the three cases in which the partner left gonad rudiment survived, it differentiated into a left ovary. Although there are slight variations in the structure of the right ovaries, they are within the range found characteristic for the normal right ovary. Furthermore, they differentiate equally well when combined with a testis and grown on a male host as when combined with ovarian tissue and grown on a female host.

2. *Right testes*.—Four of the eleven testes which differentiated from right gonad rudiments were combined with left ovarian tissue from donors ranging in age between 13 days' incubation and 75 days' post-hatching. Two grew on male, and two on female, hosts. One testis was combined with a right ovary from an embryo of $17\frac{1}{2}$ days' incubation and grew on a female host, and the remaining six were associated with testicular tissue from embryos of 18 days' incubation or from chicks as old as 80 days. Four of these developed on female, and two on male, hosts.

Testes which arise from right gonad rudiments in combination grafts are similar in structure to those arising in grafts of right rudi-

ments transplanted alone, and closely resemble those described by Willier (1927). Their essential structural components consist of a surrounding tunica albuginea which is continuous with the connective tissue stroma containing the sexual cords. These cords are composed of supporting epithelial cells and primordial germ cells.

3. *Right gonad-like bodies*.—Although the right gonad rudiment differentiates in the majority of combination grafts into gonads of specific sex, in five such grafts it gave rise to gonad-like bodies whose sex could not be definitely ascertained. These vary considerably in structure. They are always small and generally poorly organized. Some have a definite shape and are rather sharply delimited from other structures in the graft, in which case they present a compact appearance and consist of "nests" of epithelial cells and germ cells imbedded in supporting connective tissue. Others can be considered little more than scattered groups of epithelial and primordial germ cells which occur in the mesenchyme of the graft in the vicinity of the mesonephric body.

It is necessary to investigate the conditions under which these right gonad-like bodies develop, to determine whether their origin may be related to the sex of the associated gonad or to the sex of the host.

Of the five, one was combined with a right ovary from an embryo of 15 days' incubation and grown on a male host; three with a pair of testes from 16-day embryos, on male hosts; and one with left ovarian tissue from an 80-day-old hen on a female host. Unfortunately, four of the five left gonad rudiments which served as controls for these grafts were lost, because of death of the host, and the originally determined sex of four of the five right rudiments in the combination grafts was therefore not ascertained. In the fifth case the right rudiment, which failed to produce a gonad of specific sex, had a female-determining constitution. It was associated with two testes from a 16-day embryo and grew on a male host. Little direct evidence is available, therefore, which points to a positive correlation between the sex of the associated gonad and the failure of the rudiment to differentiate specifically as to sex. Certain indirect evidence, however, indicates that the origin of these right gonad-like bodies is not due to sex hormones secreted by either the associated gonad or the

sex glands of the host. (1) Right gonad rudiments, originally determined female, develop into typical right ovaries when associated in the same graft with testicular tissue and grown on male hosts (see Plate I, Fig. 1). (2) Testes having a normal appearance arise from right gonad rudiments transplanted with left ovarian tissue and grown on female hosts (see Plate I, Fig. 2). (3) Four morphologically "indifferent" right gonads which were transplanted alone as controls developed into gonad-like bodies of undetermined sex, similar in structure to those occurring in the combination grafts. Of these four cases, two developed on male, and two on female, hosts.

E. DIFFERENTIATION OF THE LEFT "INDIFFERENT" GONAD

Ninety-two grafts were obtained in which a morphologically undifferentiated left gonad developed in combination with older ovarian or testicular tissue. Forty-one differentiated into left testes and forty into left ovaries. The remaining eleven gave rise to gonad-like structures which are undistinguishable as to sex.

1. *Left testes*.—Of the forty-one testes, six were combined with left ovarian tissue obtained from donors ranging in age between 18 days' incubation and 80 days' post-hatching. Two grew on female, and four on male, hosts. Ten were associated with right ovaries from embryos incubated $12\frac{1}{2}$ –18 days. Of these, five developed on female, and five on male, hosts. Twenty-five developed in grafts with testicular tissue from donors ranging in age between $12\frac{1}{2}$ days' incubation and 51 days' post-hatching. Of these, fourteen grew on female hosts, the remaining eleven on male hosts.

Twenty-five of the forty-one right rudiments which served as controls to the left rudiments in combination grafts survived, each of which differentiated into a testis, thus proving that their partners in the combination grafts differentiated according to their originally determined male constitution.

The testes derived from left gonad primordia in combination grafts are similar in every respect to those already described which differentiated from right gonad rudiments grown under similar conditions. Moreover, they are identical in histological structure to those arising from either left or right gonad rudiments which were transplanted alone to the chorioallantoic membrane.

2. *Left ovaries*.—Left gonad rudiments, whose originally determined sex is female, exhibit wide variation in their capacity for self-differentiation in combination grafts. Of the forty left ovaries, twenty-one are structurally similar to the normal left ovary of a 13- or 14-day chick embryo, and nineteen show structural abnormalities in varying degrees.

Of the twenty-one which show a typical left ovarian structure, five developed in grafts with older left ovarian tissue obtained from embryos of 14–18 days' incubation and from hatched fowl as old as 112 days. Four such grafts developed on female, and one on a male, host. Five developed in combination with right ovaries from 13- to 18-day chick embryos; four on female, and one on a male, host. Eleven developed in grafts with testicular tissue (donors ranging in age from 16 days' incubation to 48 days after hatching), seven of which developed on female, and four on male, hosts.

In these twenty-one cases the ovary is composed of well-differentiated cortical and medullary elements (Plate I, Fig. 3), both of which contain epithelial and germ cells. A few cortical cords are in the process of invagination and remain attached to the well-developed columnar germinal epithelium, while others have lost connection with their point of origin. The medulla shows an outer compact area and an inner area composed of much-distended cords. Such grafted left ovaries have been described in detail by Willier (1927). The histological picture is remarkably similar to that of a normal left ovary of the same age.

Although left ovaries closely resembling the normal may develop from left "indifferent" gonads in combination grafts, nineteen left ovaries developing from left gonad rudiments in similar grafts show rather striking deviations from the normal structure.

Of these nineteen, one was associated with left ovarian tissue from an 80-day-old hen and grown on a female host. Three were combined with right ovaries from 18-day embryos and grown on male hosts. The remaining fifteen were combined with testicular tissue from donors ranging in age between 16 days' incubation and 48 days' post-hatching. Fourteen of these developed on male, and one on a female, host. Sixteen of the nineteen control right gonads survived and differentiated into right ovaries, thus proving that their

partner left rudiments in the combination grafts were originally determined as female. These atypically formed left female sex glands have certain characteristics in common: (1) small size; (2) absence of a columnar-celled germinal epithelium; (3) reduction or absence of cortical cords; and (4) compact masses of cellular cords, apparently medullary, which contain a greater number of germ cells than is characteristic of the medulla of normal left ovaries of corresponding age. In general, their appearance is similar to that of right ovaries derived from the opposite right "indifferent" gonad transplanted alone as a control.

The atypical left ovaries show individual variation in structure, and therefore a number of them will be described separately. One which was combined with left ovarian tissue from an 80-day-old hen and grown on a female host shows, through four or five serial sections, a germinal epithelium composed of columnar cells from which one or two cortical cords have invaginated. The greater portion of its surface, however, is devoid of a typical germinal epithelium and is covered by a layer of flattened epithelial cells beneath which no cortical elements have been formed. The medulla, which composes nearly the entire gland, consists of a connective tissue stroma in which are imbedded the medullary cords. Primordial germ cells are abundant, typically arranged in "nests." That the gonad's originally determined sex was female is proved by the fact that its partner right gonad, transplanted alone, developed into a typical right ovary.

One of the three modified left ovaries which were combined with right ovaries obtained from chicks near hatching and grown on male hosts is illustrated in Plate I, Figure 4. The germinal epithelium is composed of flattened cells, and cortical cords are completely lacking. Many primordial germ cells, arranged in "nests," are present. Two or three open spaces, bounded by a layer of flattened epithelial cells, suggest a distention of medullary cords. In some of these open spaces are found primordial germ cells, either singly or in small groups. The ovary has, in general, a compact appearance and is found to consist apparently of medullary elements only. Unfortunately, the control right gonad was lost, owing to death of the host following operation. However, its structure so closely corresponds to other

atypical left ovaries that little doubt exists that the left rudiment which gave rise to it was originally determined female.

Of the fifteen atypical left ovaries which developed in combination with testicular tissue, three may be described to illustrate the range of variation in structure which they exhibit. A left gonad rudiment combined with a testis from an 18-day embryo and grown on a male host gave rise to the left ovary illustrated in Plate II, Figure 5. The gland is small and covered by a distinct germinal epithelium composed of cuboidal epithelial cells and a few germ cells. From this germinal epithelium several cellular cords have invaginated which are probably cortical cords, although they are more slender and contain fewer well-differentiated germ cells than do normal cortical cords. The deeper portion of the gland is composed of nests of cells which contain both epithelial and primordial germ cells. These structures probably represent the medullary component of the ovary.

The left female sex gland, shown in Plate II, Figure 6, developed in a graft with two testes obtained from a 17-day embryo. It was grown on a male host. It has a germinal epithelium covering only about one-third of its free surface, from which no cortical cords have invaginated. The remainder of the gland is comprised of medullary cords imbedded in a connective-tissue stroma. These medullary cords are made up of epithelial cells and a few primordial germ cells.

A left ovary which is extremely abnormal in structure was derived from an "indifferent" left gonad primordium combined with a testis from an 18-day chick embryo and grown on a male host (Plate II, Fig. 7). It is small and shows a germinal epithelium composed of flattened cells; no cortical cords are present. The gland is composed entirely of medullary cords which are made up of two types of cells, epithelial and primordial germ cells, the latter greatly predominating. This fact gives the gland the appearance, when examined under low magnification, of being composed almost entirely of "nests" of primordial germ cells. No distention of the medullary cords occurs in this left ovary. The control graft contains a well-differentiated right ovary.

It is evident, then, that left ovaries arising from "indifferent" left gonad rudiments combined with testicular tissue may appear quite

PLATE II

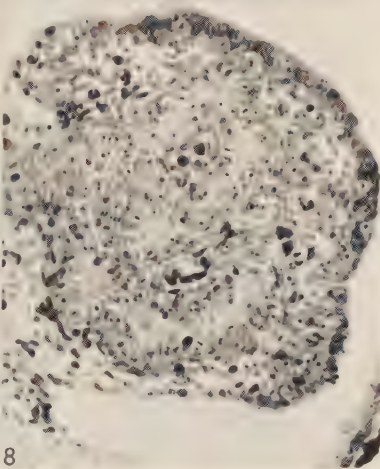
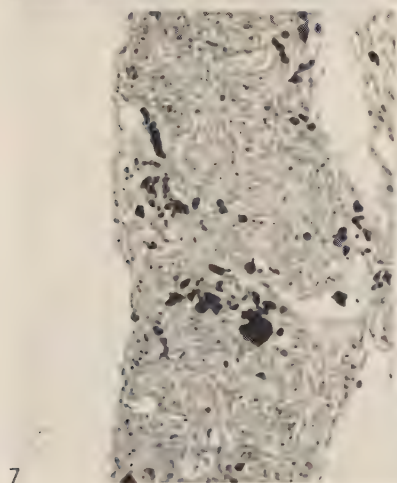
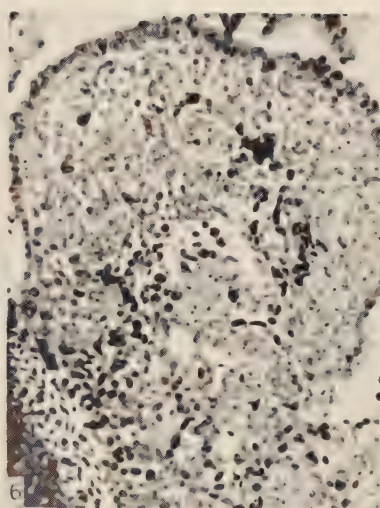
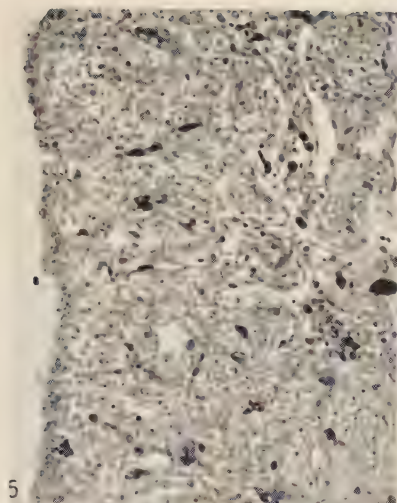
FIG. 5.—Section of a slightly modified left ovary which developed in the same graft with an 18-day embryonic testis. The host was a male. $\times 500$.

FIG. 6.—Section of a modified left ovary derived from a left gonad rudiment which was associated with two testes obtained from a 17-day embryo; male host. $\times 525$.

FIG. 7.—Section of an abnormal left ovary which developed from a left rudiment combined with an 18-day testis and grown on a male host. $\times 500$.

FIG. 8.—Section of a gonad-like body possessing male sex cords and a germinal epithelium. It arose from a left gonad rudiment combined with a right ovary of a 16-day embryo. The host was a male. $\times 525$.

PLATE II



normal or may show structural modification in varying degrees. In those less strikingly atypical in structure a germinal epithelium may be present from which a very sparse proliferation of cortical cords has occurred (one case); those showing greater modification may possess a germinal epithelium consisting of columnar or cuboidal cells but no cortical cords (two cases); finally, the left ovary may consist of medullary elements only, both germinal epithelium and cortical cords being entirely lacking (twelve cases). This tendency for left gonad rudiments having a female-determining constitution to develop in an atypical manner in combination grafts stands out in marked contrast to the capacity for typical differentiation exhibited by right rudiments which were originally determined female and by rudiments having a male-determining constitution (either right or left). It will be noted in Table I that, of the seventeen left ovaries which differentiated in combination grafts grown on female hosts, fifteen possess a typical structure and two are atypical. Of the twenty-three left ovaries in combination grafts supported by male hosts, only six are typical in structure, while seventeen present an atypical histological picture. These data, examined statistically, give a difference of 62.1 per cent, or 3.88 times the standard error. Only 12.5 per cent of the left ovaries which differentiated in grafts containing testicular tissue and which grew on female hosts were abnormal, while similar left ovaries also associated with testicular tissue but grown on male hosts present an atypical histological picture in 77.7 per cent of the cases. It would appear, therefore, that even though the sex of the differentiated gonad tissue in the same graft is disregarded, the sex of the host must be considered a possible factor influencing the capacity of the left rudiment, originally determined female, to differentiate into a typical left ovary.

3. *Left gonad-like bodies*.—Of the eleven gonad-like bodies derived from left gonad rudiments, one was combined with a left ovary from a 19-day embryo and grown on a male host. Another developed, with a 16-day embryonic right ovary, on a male host; and nine were found in grafts with testicular tissue from donors ranging in age between 14 days' incubation and 80 days' post-hatching. Of these nine, four developed on female, and five on male, hosts.

With one exception, these gonad-like bodies are very similar in structure to those which develop from right gonad rudiments. The one exceptional left gonad, which developed in a graft with a right ovary, on a male host, has definite structural peculiarities (Plate II, Fig. 8). Most of its free surface is covered with a germinal epithelium composed of columnar cells between which occur primordial germ cells. The greater portion of the gland is composed of well-differentiated sex cords which closely resemble, structurally, those of a normal testis of a chick of approximately 14 days' incubation. The primordial germ cells are distributed irregularly throughout the sex cords, while the supporting epithelial cells are arranged radially around the long axis of the cord, their oval nuclei showing a linear arrangement along the periphery. Although the sexual cords are typically testicular in appearance, the germinal epithelium is more characteristic of a left ovary. Unfortunately, the control graft containing the partner right gonad was not recovered.

The originally determined sex of four of these eleven gonad-like bodies was ascertained. These four, originally determined male, were combined with testicular tissue from donors aged 50 days after hatching. Two of them developed on female, and two on male, hosts.

Three similar gonad-like bodies developed from left gonads transplanted alone. Two of these grafts developed on female, the remaining one on a male, host.

F. DEVELOPMENT OF THE OLDER GONAD COMPONENT IN THE GRAFT

Testes isolated from chicks just before, or a few days after, hatching become completely vascularized following transplantation, and continue to develop in essentially a normal manner (Plate I, Fig. 3). Large transplants of testicular tissue from juvenile fowl (40-90 days of age) frequently fail to become completely vascularized rapidly enough to prevent necrosis at their center. Even in those cases where degenerative changes are evident centrally, a large number of apparently healthy and well-differentiated semeniferous tubules are present around the periphery of the transplant.

All right ovaries contributing to combination grafts were isolated from chicks during the last 4 or 5 days of the incubation period. After 8 or 9 days in the graft they are similar in appearance to nor-

mal right ovaries of chicks 4 or 5 days after hatching. They vary in size and shape, but their histological structure remains quite typically right ovarian.

Left ovarian tissue from embryonic or newly hatched chicks becomes rapidly vascularized following transplantation. Donor tissue isolated at 17 or 18 days of incubation shows well-developed Graffian follicles after 9 days on the host. Large pieces of ovarian tissue from young hens (60-112 days of age) may become necrotic centrally, owing to slow and incomplete vascularization following transplantation. The periphery of the transplant always shows many follicles containing ova, some of which have a massive accumulation of yolk, and nuclei containing formed chromosomes.

It can be definitely stated that gonad tissue from late embryonic or hatched fowl, when incorporated into a combination graft, continues to develop in a normal manner, with the exception of the central area of large transplants, which become vascularized too slowly to prevent degenerative changes.

COMBINATION GRAFTS OF SEXUALLY DIFFERENTIATED GONADS

To ascertain what influence gonads, already structurally differentiated as to sex, and of the same age, may exert upon each other in heterosexual combination in the same graft, testes and ovaries were isolated from donors of 18 days' incubation and transplanted in heterosexual pairs to a host embryo. There they developed in close proximity or in direct contact with one another for a period of 8 or 9 days.

A. COMBINATION GRAFTS OF TESTIS AND RIGHT OVARY

Seven grafts which resulted from a combination of a testis and right ovary show both gonads to have a structure essentially similar to normal gonads of corresponding age. The testis in each case continued to develop in a normal manner and exhibits a histological picture similar in most respects to the testis of a 5- or 6-day hatched chick. The semeniferous tubules are greatly elongated; and lumina, never present at the time of isolation, have developed in many of them. The right ovaries vary somewhat in structure, but not beyond the range of variation to which normal right ovaries are subject. In three cases the right ovary is a compact, definitely circumscribed

body. In two grafts it has the appearance of a network, and in one case has become infiltrated with lymphocytes. In another graft the right ovary is abnormally large and contains a considerable amount of cortical tissue. The protocol reveals, however, that areas of cortical elements were observed macroscopically on the surface of the ovary at the time of implantation.

B. COMBINATION GRAFTS OF TESTIS AND LEFT OVARY (NINE CASES)

Six grafts arising from the transplantation of left ovary with testis show both components of the graft to have continued their growth and development with little or no variation from their normal structure. In four of these six cases the testis and ovary are in direct contact, and probably had been so during the 9-day growth period on the host embryo. In the remaining three grafts the testis is quite normal in appearance and closely corresponds to the testis of a chick 5 or 6 days after hatching, but the associated left ovaries show an appreciable thinning of the cortical layer with a reduction in the number of primitive ova contained. Grafts containing normal left ovaries in the presence of testicular tissue are found on both male and female hosts. Of the three combination grafts showing ovaries with reduced cortex, two grew on male hosts and one on a female host. In two instances left ovaries of corresponding age transplanted alone on hosts (one male, one female) have shown a similar thinning of the cortical layer.

RESPONSE OF THE REPRODUCTIVE ORGANS OF THE HOST TO GONAD GRAFTS

The reproductive organs of all host embryos bearing combination, as well as control, grafts were examined under a binocular dissecting microscope for evidence of a reaction to the various types of gonad grafts. Some variations from the typical anatomical structure were observed in the reproductive organs of the hosts. These include variations in the size and shape of the gonads themselves, particularly the right ovary. The length of the persistent "cloacal stump" of the right oviduct and the diameter of the Wolffian ducts, the left oviduct, and of the shell gland are all subject to slight variations. These variations from the typical structure cannot be considered as

specific responses to gonad grafts, since they are very similar to those reported previously by Willier and Yuh (1928), who proved that they also occurred in host embryos bearing non-gonad grafts. Similar variations were observed in the structure of the reproductive organs of 18-day normally incubated chick embryos whose gonads served as donor tissue in these experiments.

"MULTIPLE-GONAD" GRAFTS

It seemed possible that lack of response to gonad grafts on the part of the hosts' reproductive organs might be due solely to the failure of the small pieces of transplanted gonad tissue to secrete sufficient sex hormone to bring about a positive response. It was hoped that grafts consisting of four or five 18-day embryonic gonads transplanted to 9-day host embryos might produce sufficient hormone to elicit a response in the reproductive organs of the hosts. Grafts consisting of as many as five testes from 18-day embryos developed for 8 or 9 days on both male and female hosts. The grafts were well vascularized, and the transplanted testicular tissue was apparently in a healthy and normal condition. The reproductive organs of the male hosts bearing such grafts were examined under the binocular dissecting microscope and found to correspond closely to those of a normally incubated 18-day male chick embryo. The sex ducts of the female hosts were normal in appearance, and histological examination of the ovaries of these hosts revealed no deviations from the normal structure. It would seem definitely established, therefore, that even large amounts of testicular tissue, from embryos just prior to hatching, are unable, when transplanted to the chorioallantoic membrane at 9 days' incubation, to affect the reproductive organs of the host.

No grafts resulting from the transplantation of three or more 18-day left ovaries were recovered, since the hosts in all such cases failed to survive the operation. Death of the host under these conditions was probably due to the large volume of tissue transplanted.

DISCUSSION

The evidence presented in the foregoing sections reveals that, with certain exceptions to be noted below, right or left indifferent gonad

rudiments, in case they have a male-determining constitution, have the capacity for self-differentiation into a testis regardless of the sex of the associated differentiated gonad and of the sex of the host. Either right or left gonad rudiments whose originally determined sex was male differentiated into testes although they were subject to hormones from an associated ovary (left or right) and grown on a female host.

Such a capacity for independent differentiation is likewise characteristic of a right gonad rudiment when it has a female-determining constitution. Such a right rudiment, although associated with testicular tissue of various ages and grown on a male host, has the capacity to differentiate into a typical right ovary.

The failure of the right ovary, developing under these conditions, to show any evidence of inversion is interesting, since it is known to have male potentialities and the capacity to transform into a testis following left ovariectomy of chicks just after hatching (Benoit, 1923*a*, 1923*b*, 1923*c*; Domm, 1927 and 1929; and others). Although the growth period of the right ovary in the combination graft is very short in comparison to the time required for the inversion following ovariectomy, it would be expected that at least the early phase of the process might be detectable histologically.

On the contrary, the left indifferent gonad primordium having a female-determining constitution exhibits considerable variation in its capacity to self-differentiate under similar developmental conditions. Twenty-one, or 53 per cent, of such left rudiments were capable of differentiating into left ovaries which closely resemble the normal left ovary of corresponding age. This capacity for self-differentiation was expressed even though the left gonad rudiment was associated with testicular tissue and grown on a male host. Nineteen, or 47 per cent, differentiated into left ovaries which show a marked reduction in size involving both medullary and cortical components, but especially the latter. In these cases the germinal epithelium becomes flattened, and cortical cords may be few or entirely lacking.

Statistical examination of the data points to a positive correlation between the occurrence of atypical left ovaries and their residence on male hosts. If such is the case, the sex of the host can be considered

as only one contributing factor in influencing the course of differentiation of the engrafted rudiment. This conclusion is based on several facts: (1) Left ovaries, quite normal in appearance, occur in grafts which also contain older testicular tissue and which develop on male hosts. (2) A modified left ovary may arise from a left gonad rudiment associated with left ovarian tissue and grown on a female host. (3) Left ovaries possessing a flattened epithelial covering and showing a marked deficiency in cortical elements arise from left gonad rudiments transplanted alone to female hosts.

Sixteen gonad rudiments (eleven left and five right) gave rise in combination grafts to gonad-like bodies whose sex cannot be definitely ascertained. Since the originally determined sex of the gonad rudiment is known in only five of these cases the data are inconclusive on the question of a possible inhibition of the rudiment's self-differentiating capacity due to hormones secreted by the associated gonad or by the host. The following evidence strongly indicates that these gonad-like bodies do not owe their origin to gonad rudiments developing under the influence of antagonistic sex hormones: (1) Indifferent gonad rudiments which have a male-determining constitution give rise to gonads of unspecific sex, although subject to hormones from associated testicular tissue and from a male host. (2) Similar gonad-like structures whose sex cannot be definitely ascertained arise from gonad rudiments transplanted alone and grown on either male or female hosts.

The originally determined sex of the left gonad which possesses structural characteristics of both a testis and an ovary (Plate II, Fig. 8) was not definitely ascertained, because of the loss of its control partner. It is highly probable, however, that the rudiment possessed a male-determining constitution and that the germinal epithelium persisted for several days after male sex cords had differentiated. Such a persistence of the germinal epithelium on the surface of the left testis of a chick embryo of 12 days' incubation was reported by Laulanié (1886), and the left testis of a late duck embryo described by Burwell (1931) exhibited a similar condition. Persistence of the germinal epithelium on the surface of the right testis has not been reported.

Willier, Gallagher, and Koch (1935) have experimentally stimulated the proliferation of cortex on the surface of the left testis of the chick by injecting theelin, theelol, or male hormone (urine derivative) into the albumin of the incubating egg. The right testis, on the contrary, does not respond to the hormones injected. These investigators attribute the difference in response between the two testes to the presence of a germinal epithelium which develops only on the left testis.

Since the left gonad shown in Plate II, Figure 8, possesses a germinal epithelium but no cortical cords, its origin can perhaps be more correctly attributed to the normal persistence of the germinal epithelium than to a response to hormones.

Although, based on statistical evidence, a positive correlation exists between the occurrence of atypical left ovaries and their residence on male hosts, we are faced with the fact that similar modified left ovaries arise in grafts which are grown on female hosts. It seems necessary to assume, therefore, that some other factor may strongly contribute to this disturbance of the normal course of differentiation which results in modified left ovaries and in the occurrence of gonad-like bodies whose sex cannot be ascertained. This factor may be purely a mechanical one.

Buyse (1935) finds that indifferent gonads of the rat embryo, when transplanted to a subcapsular position in the kidney of an adult male rat, give rise to: (1) testes essentially normal in appearance, (2) ovaries approximating normal structure, (3) ovaries which may show a marked deficiency in cortical elements, (4) ovotestes, and (5) gonads whose sex cannot be ascertained. That this modification is not a "free-martin" effect was proved when similarly modified ovaries were recovered which had arisen from undifferentiated gonad rudiments transplanted to female hosts. Buyse concludes that the modification is probably due to a high susceptibility of the cortical tissue to unfavorable mechanical factors in the graft.

The cortical reduction exhibited by the modified left female gonads in these experiments may result from unfavorable developmental mechanics operating in the graft to which the cortex is more susceptible than is the medullary tissue. In case the mechanical factors are sufficiently unfavorable, normal growth and differentiation

may be inhibited to such an extent that lack of structural organization makes identification of the sex of the gland impossible.

With the exception of a possible influence exerted on the differentiating left female rudiment by its male host, the lack of specific response to hormones on the part of the gonads in the various types of graft and host-graft combinations is striking. Gonad rudiments originally determined male and right rudiments originally determined female, when transplanted in juxtaposition with testes and ovaries from embryonic chicks and grown on either male or female hosts, evidently may differentiate according to their original plan of organization, irrespective of hormones to which they are subjected. Since 28.5 per cent of the left ovaries having a typical structure arose in combination grafts which developed on male hosts, a specific response of the left female rudiment to hormones secreted by the male host may even be questioned.

Ovarian and testicular tissue, known to be producing hormone at the time of transplantation, show no greater capacity for influencing the direction of differentiation of the gonad rudiment in the same graft than do embryonic ovaries and testes. The fact is thoroughly appreciated that no proof exists that the older gonad tissue continues to secrete hormone when transplanted to the membrane. Nevertheless, it is necessary to point out that, in case hormone secretion continues, the hormone is either produced in too small a quantity or does not have the capacity to influence the direction of sex differentiation in the associated gonad rudiment.

Ovaries and testes differentiated as to sex at the time of implantation develop in close proximity or in contact in heterosexual combination for a period of 8 or 9 days and continue to develop in essentially a normal manner.

These facts are interesting when viewed in the light of modifications which occur in the gonads of heterosexual pairs of certain parabiotic amphibia, owing, according to Witschi (1931 and 1934), to an antagonism between cortical and medullary elements which are in close proximity. The data would seem to justify the conclusion that in the chick no such direct local effect is exerted by one gonad upon another of opposite sex as the result of close association in space over a 9-day growth period.

The lack of response on the part of the reproductive organs of the host embryo to the various types of gonad grafts developing on the chorioallantoic membrane corroborates the findings of Willier (1925 and 1927), Kemp (1925 and 1927), Willier and Yuh (1928), and others. Even "multiple-testis" grafts interposed in the blood circulation of the host embryo failed to modify the structure of its reproductive glands and ducts. This does not necessarily imply that these organs are not capable of a response to large quantities of male hormone, but that the dosage necessary for such a response is greater than that which may be supplied by transplantation of the living testicular tissue to the membrane.

SUMMARY

1. Morphologically undifferentiated chick gonad rudiments and older ovarian or testicular tissue were transplanted together on the chorioallantoic membrane of 8- or 9-day embryos, to determine what influence the sex of the associated gonad or of that of the host might have upon the rudiment's capacity to self-differentiate. To ascertain the originally determined sex of the gonad rudiment in the combination graft, its partner gonad from the same donor embryo was transplanted alone to a second host.

2. Right and left rudiments originally determined male and right rudiments having a female-determining constitution were found to possess the capacity to self-differentiate into testes and right ovaries, respectively, regardless of the sex of the associated gonad and of that of the host.

3. Left rudiments, originally determined female, gave rise in such grafts to left ovaries, 53 per cent of which correspond closely to the normal. The remaining 47 per cent exhibit structural modifications involving principally the cortical elements. Although a modified left ovary may develop in combination with left ovarian tissue on a female host and with testicular tissue on a male host, statistical evidence points to a positive correlation between atypical left ovaries and their residence on a male host.

4. Five right and eleven left gonad rudiments in combination grafts gave rise to gonad-like bodies of unspecific sex. These were associated with either ovarian or testicular tissue and developed on

both male and female hosts. Similar structures arise from gonad rudiments transplanted alone to either male or female hosts.

5. Some factor other than the sex of the associated gonad or the sex of the host evidently contributes to the occurrence of the modified left ovaries and the gonad-like bodies whose sex cannot be ascertained. This factor is probably unfavorable developmental mechanics operating in the graft.

6. No evidence was found of a local effect of cortical and testicular (or medullary equivalent) elements upon each other. This is true for combinations of differentiated gonad tissue of opposite sex, as well as for differentiated and undifferentiated gonad combinations.

7. Gonad tissue known to be producing hormone at the time of transplantation is no more effective in altering the course of differentiation of the gonad rudiment in the same graft than is gonad tissue from embryos.

8. Although slight variations in structure occur in the reproductive organs of the host embryos, they are all within the range found characteristic of the normal embryo.

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